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NEW UTILITY PATENT APPLICATION TRANSMITTAL

(to be used for new applications only)

Attorney Docket Number	
First Named Inventor	ASHOK K. SHUKLA
Total Pages in this Submission	Text 15 Pages Drwg 5 Sheets + Forms

JC860 U.S. PTO 10/26/00

09/696770 U.S. PTO Jd921 10/26/00

APPLICATION ELEMENTS

*Notice Checklist items mentioned under **Application Elements** section construct a new utility patent application Please refer to MPEP Sections 506, 601, (37CFR 1 77, 1 53, 35 USC 111, 112, 113) for detailed explanation regarding completeness of an original patent application.*

1. [X] Fee Transmittal Form (prescribed filing fee(s))
2. Specification
   - [X] Title of the Invention
   - [ ] Cross References to Related Applications *(if applicable)*
   - [ ] Statement Regarding Federally-sponsored Research/Development *(if applicable)*
   - [ ] Reference to Microfiche Appendix *(if applicable)*
   - [X] Background of the Invention
   - [X] Brief Summary of the Invention
   - [X] Brief Description of the Drawings *(if drawings filed)*
   - [X] Detailed Description
   - [X] Claim or Claims
   - [X] Abstract of the Disclosure
3. [X] Drawing(s) *(when necessary as prescribed by 35 USC 113)*
4. [X] Executed Declaration
5. Genetic Sequence Submission *(if applicable, all must be included)*
   - [ ] Paper Copy
   - [ ] Computer Readable Copy
   - [ ] Statement Verifying Identical Paper and Computer Readable Copy

ACCOMPANYING APPLICATION PARTS

6. [ ] Assignment Papers
7. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
8. [ ] Computer Program in Microfiche
9. [ ] English Translation Document *(if applicable)*
10. [ ] Information Disclosure Statement/PTO-1449 [ ] Copies of IDS Citations
11. [ ] Petition Checklist and Accompanying Petition
12. [ ] Preliminary Amendment
13. [ ] Proprietary Information
14. [ ] Return Receipt Postcard
15. [X] Small Entity Statement
16. [ ] Additional Enclosures *(please identify below):*

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual name	ASHOK K. SHUKLA
Signature	
Date	10/26/00

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Application Number			Class		Independent Claims		
Date of Receipt	Application Type		GAU		Total Claims		
	Filing Date		Foreign Filing License?		Drawing Sheets		
	Small Entity		Foreign Address?		Special Handling?		

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FEE TRANSMITTAL

Complete If Known	
Application Number	
Filing Date	10/26/00
First Named Inventor	ASHOK K SHUKLA
Group Art Unit	
Examiner Name	
Attorney Docket Number	

TOTAL AMOUNT OF PAYMENT ($) 345.00

METHOD OF PAYMENT (check one)

1. ☐ The Commissioner is hereby authorized to charge indicated fees and credit any over payments to:

Deposit Account Number

Deposit Account Name

☐ Charge Any Additional Fee Required Under 37 CFR 1.16 and 1.17

☐ Charge the Issue Fee Set in 37 CFR 1.18 at the Mailing of the Notice of Allowance, 37 CFR 1.311(b)

2. ☒ Payment Enclosed:

☒ Check ☐ Money Order ☐ Other

FEE CALCULATION (fees effective 10/01/96)

1. FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	770	201	385	Utility filing fee	345.00
106	320	206	160	Design filing fee	
107	530	207	265	Plant filing fee	
108	770	208	385	Reissue filing fee	
114	150	214	75	Provisional filing fee	

SUBTOTAL (1) ($)

2. CLAIMS

		Extra		Fee from below		Fee Paid
Total Claims	-20 =		X		=	
Independent Claims	- 3 =		X		=	
Multiple Dependent Claims			X		=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	22	203	11	Claims in excess of 20
102	80	202	40	Independent claims in excess of 3
104	260	204	130	Multiple dependent claim
109	80	209	40	Reissue independent claims over original patent
110	22	210	11	Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 345.00

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet.	
139	130	139	130	Non-English specification	
147	2,460	147	2,460	For filing a request for reexamination	
112	900*	112	900*	Requesting publication of SIR prior to Examiner action	
113	1,790*	113	1,790*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for response within first month	
116	390	216	195	Extension for response within second month	
117	930	217	465	Extension for response within third month	
118	1,470	218	735	Extension for response within fourth month	
119	300	219	150	Notice of Appeal	
120	300	220	150	Filing a brief in support of an appeal	
121	260	221	130	Request for oral hearing	
138	1,470	138	1,470	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive unavoidably abandoned application	
141	1,290	241	645	Petition to revive unintentionally abandoned application	
142	1,290	242	645	Utility issue fee (or reissue)	
143	440	243	220	Design issue fee	
144	650	244	325	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	230	126	230	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	770	246	385	Filing a submission after final rejection (37 CFR 1.129(a))	
149	770	249	385	For each additional invention to be examined (37 CFR 1.129(b))	

Other fee (specify) _____

Other fee (specify) _____

SUBTOTAL (3) ($)

* Reduced by Basic Filing Fee Paid

SUBMITTED BY

				Complete (if applicable)	
Typed or Printed Name	ASHOK K SHUKLA			Reg. Number	
Signature		Date	10/26/00	Deposit Account User ID	

PTO/SB/09 (10-96)
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Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(b))—INDEPENDENT INVENTOR

Docket Number (Optional)

Applicant or Patentee: ASHOK K. SHUKLA, MUKTA M. SHUKLA & AMITA M. SHUKLA

Application or Patent No.: NEW APPLICATION

Filed or Issued: 10/26/00

Title: Mucin - Biomolecules Complexes for Transfection

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees to the Patent and Trademark Office described in:

- [X] the specification filed herewith with title as listed above.
- [ ] the application identified above.
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Each person, concern, or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

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Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
MUKTA M. SHUKLA		
Signature of inventor	Signature of inventor	Signature of inventor
10/26/00		
Date	Date	Date

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Filed or Issued: 10/26/00

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NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
ASHOK K SHUKLA		
Signature of inventor	Signature of inventor	Signature of inventor
10/26/00		
Date	Date	Date

PTO/SB/09 (10-96)
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VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(b))—INDEPENDENT INVENTOR

Docket Number (Optional)

Applicant or Patentee: ASHOK K. SHUKLA, MUKTA, M. SHUKLA & AMITA M. SHUKLA

Application or Patent No.: NEW APPLICATION

Filed or Issued: 10/26/00

Title: Mucin - Biomolecule Complex for Transfection.

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees to the Patent and Trademark Office described in:

- [X] the specification filed herewith with title as listed above.
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AMITA M. SHUKLA		
NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
Amita Shukla		
Signature of inventor	Signature of inventor	Signature of inventor
10/26/00		
Date	Date	Date

TITLE: **Mucin-Biomolecules Complex for Transfection**

INVENTORS: Ashok K. Shukla, Mukta M. Shukla and Amita Shukla, 10423 Popkins Court, Woodstock, MD 21163.
Ph. (410) 465-2212

FIELD OF THE INVENTION

In the present invention we describe a new method for the formation of a mucin-biomolecules complex, such as a mucin-DNA (deoxyribonucleic acid) complex and the application of such a complex for the transport of DNA, RNA (ribonucleic acid) and other biomolecules into cells. Transfection is the introduction of a DNA molecule into a eukaryotic cell, usually followed by the expression of one or more genes in the newly introduced DNA. The mucin-DNA complex described in the present invention can be used to perform transfection of DNA, as well as, the introduction of RNA and other larger biomolecules into cells. Since effective transfection, especially in *in vivo* systems is still limited by the methods currently available, the mucin-DNA complex, as described in the present invention, presents a novel and significantly improved method for performing transfection and ensuring the effective transmission of DNA into cells and the expression of genes in transfected DNA.

BACKGROUND OF THE INVENTION

Transfection, or the introduction of a DNA molecule into a eukaryotic cell, usually followed by the expression of one or more genes in the newly introduced DNA, represents one of the most important steps in genomics research and gene therapy. While methods for isolating DNA for transfection have improved significantly, effective methods for transfecting isolated DNA strands, especially in *in vivo* systems, are the limiting factor for progress in gene therapy. A number of transfection methods currently exist, yet each one of them is limited in the scope of its application and each presents certain disadvantages.

Current transfection methods include calcium phosphate precipitation, the use of a cationic lipid - DNA complex, electroporation and the use of viral vectors. Yet, calcium phosphate precipitation does not always yield high levels of transfection in cells. Cationic lipids, used in a complex, are often toxic to cells and thus ineffective for *in vivo* tranfection for gene therapy. Electroporation is a method where very high voltage levels are used to transport DNA into cells. Since DNA is highly negatively charged, the application of such an electric current allows for the passage of DNA into cells. Yet, this method cannot be used for *in vivo* transfection. Also, at high voltage levels the death rate of cells is significantly higher, even further limiting the scope of this method.

In viral vector transfection, the DNA to be tranfected is first introduced into the DNA of a virus. The virus, in turn, then injects its DNA, including the desired

tranfection DNA, into a host cell. Although this method can be used in *in vivo* systems, one of its main disadvantages is that the virus can transform itself or its DNA and thus create undesirable side effects such as harmful infection of the host or undesired transformations to host DNA. The utility of this method is thus also significantly limited for gene therapy.

Since current transfection methods are so limited in their scope and utility there is strong need for a non-toxic method for in vivo transfection that has high success rates for transporting DNA into cells and that minimizes harmful side effects. Also, since the specificity of current methods is very limited, a more specific method for transfection is needed to ensure that desired DNA fragments are introduced into specific target cells. The present invention describes a mucin-DNA complex which represents a novel and highly effective method for transfection.

Mucins are glycoproteins with a very high molecular weight (usually more than 1 million Daltons). Mucins are generally about 60 percent or more carbohydrate by composition and water soluble. The carbohydrate molecules are generally attached as chains to the backbone of the proteins. Since carbohydrates are generally linear molecules the resulting structure can be likened to that of a comb, with the carbohydrate molecules forming individual prongs. When such a mucin molecule is combined with isolated strands of DNA a complex is formed in which the carbohydrate and protein molecules of mucin entangle the DNA strands to form a mucin-DNA complex. Said mucin-DNA complex can be precipitated using a number of different

methods. Said complex can also be re-suspended and centrifuged to extract desired components of the complex.

The mucin-DNA complex as described in the present invention offers a number of advantages over currently available methods since said complex is:

1. non-toxic;
2. very specific since the choice of outer molecules on the mucin component of the complex can be used to specify which target cells will recognize said complex;
3. easy to create;
4. and, free of harmful side effects such as those resulting in cell toxicity.

Said mucin-DNA complex thus represents an effective method for transfection and thus presents a highly effective, new method for performing gene therapy.

The various features of novelty, which characterize the present invention, are pointed out with particularity in the claims annexed to and forming a part of this disclosure. For a better understanding of the invention, its advantages and objects, reference is made to the accompanying drawings and descriptive matter in which a preferred embodiment of the invention is illustrated.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and still other objects of this invention will become apparent, along with various advantages and features of novelty residing in the present embodiments, from study of the following drawing, in which:

Figure 1 is an expanded view of one embodiment of mucin (Figure 1(a)) and DNA (Figure 1(b)) molecules in chain form, according to the present invention.

Figure 2 is an expanded view of one embodiment of the mucin-DNA complex, according to the present invention.

Figure 3 is an expanded view of one embodiment of the mucin-DNA complex after transfection into a target cell, according to the present invention.

Figure 4 is an expanded view of one embodiment of a molecule (sialic acid) from the carbohydrate chain of mucin with modification at the carboxyl group in Figure 4(a) and modification at the N-acetyl group in Figure 4(b), according to the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Figure 1(a) shows a mucin molecule (1) with a protein backbone (2) and carbohydrate chains (3) attached to said backbone (2). Said mucin molecule (1) may be any type of mucin molecule with a structure that may or may not resemble the structure and outline in Figure 1(a). Figure

1(b) shows a linear representation of a DNA strand (5). As shown, the backbone of the DNA strand (6) contains negatively charged molecules.

Figure 2 shows an entangled complex comprised of mucin and DNA molecules to form a mucin-DNA complex. As shown in Figure 2, the protein backbone (2) and carbohydrate chains (3) of the mucin molecule are intertwined with the strands of the DNA molecule (5). When mucin and DNA are present in a complex as shown in Figure 2, the individual strands of the respective molecules cannot be separated easily, creating the tangled complex shown in the figure. When a precipitating agent such as ethanol, tannins or an aqueous solution is used mucin and DNA both precipitate, forming a complex. The resulting mucin-DNA complex can be re-suspended in solution by agitation, shaking or ultrasonication, and can be re-precipitated again when centrifuged. Said mucin-DNA complex, as shown in figure 2, can be purified through centrifugation and washing with buffer.

The DNA strand (5) may be of any length and may be present in any configuration. Although Figure 2 shows a DNA strand, the mucin molecule can also be used to form complexes with other biomolecules such as RNA, to form mucin-RNA complex or certain proteins to form mucin-protein complexes. In the latter example, RNA or said proteins are transported into a cell using the method of the present invention. The biomolecules bound to mucin may be any biomolecules from the group consisting of, but not limited to, DNA, RNA, nucleic acids, proteins, peptides,

antibodies, glycolipids, glycoproteins, natural, synthetic and modified polymers, or any combination thereof.

Figure 3 shows a cell into which the mucin-DNA complex has been transported. Thus, the combination of DNA with mucin is effective for transporting strands of DNA (5) into desired target cells (7). As shown in Figure 3, the mucin molecule components, the protein backbone (2) and complex carbohydrate strands (2) may break down into smaller particles upon entry into the interior of the cell (8), but the DNA strand (5) is transported intact, for the most part and results into the subsequent incorporation of the introduced DNA into the existing DNA of said cell (7).

The specificity of target cells for transfection can be controlled through specific modifying molecules on the mucin component of the mucin-DNA complex, as shown in Figure 4. Figure 4(a) shows a carbohydrate molecule, sialic acid (9), where an ester group has been added to the carboxyl group (10), whereas Figure 4(b) shows the same carbohydrate molecule (9) with modification at the N-acetyl group (11). Any type of modification can be performed on either the protein (2) or carbohydrate (3) components of said mucin molecule, as is relevant to a given set of cells targeted for transfection. Said modifications include the addition, removal or alternation or carbohydrate or protein components of mucin in said mucin-DNA complex.

One of the main advantages of the mucin-DNA complex, as shown in Figure 2, is that mucin is a natural product and is non-toxic. For successful transfection for *in vivo* gene therapy mucin can be isolated from the same patient

who will be the recipient of DNA during transfection. This is highly useful since it prevents the risk of toxicity to the patient. Also, as shown in Figure 3, mucin can be chemically modified. Furthermore, mucin can also be used in natural or chemical form and can be purified or modified using any chemical or enzymatic methods.

Mammalian organisms and cells represent a significant source of mucin, but any other organisms or cells, including bacteria or plants can also be used as mucin sources. Once mucin is obtained from a desired source it can be purified by chromatographic methods or by precipitation and re-suspension. Alternately, mucin can also be used in 'as-is' form from the source, without further purification.

Most mammalian mucin molecules have sialic acids as terminal molecules. The total or partial removal of sialic acid molecules, either enzymatically or chemically, can further enhance the binding of DNA to the mucin. Since both DNA and sialic acids are highly negatively charged, the two types of molecules would repel each other. With the removal of sialic acid, DNA binds to mucin more easily. Furthermore, the removal of sialic acid also enhances the endocytosis of the mucin-DNA complex. Endocytosis is the process whereby a cell adheres a certain molecule or complex to its exterior cell membrane and then engulfs it to introduce that molecule or complex into the interior of the cell. When sialic acid is removed from mucin, galactose molecules become the terminal molecules of the mucin carbohydrate chains. Galactose is often better

recognized by cell surface molecules for endocytosis of the mucin-DNA complex.

Thus, modifications, such as the removal of sialic acid, may be advantageous and could be performed on the native mucin to enhance its transfection capabilities. Alternately, the negative charges on sialic acid could be suppressed by the esterfication (addition of an ester group) to the carboxyl group (10) of sialic acid (9), as shown in Figure 4(b). The subsequent formation of an ester group (ethyl or methyl) would remove the negative charge from sialic acid. Furthermore, sialic acid has an N-acetyl group at C-5 (11), as shown in Figure 4(a). The removal of this acetyl group would confer a positive charge on that component of the sialic acid molecule, thus increasing its binding to the negatively charged DNA. Alternately, both the acetyl group and the hydrogen atom at the nitrogen atom can be replaced with alkyl groups, such as $-CH_3$, $-C_2H_5$. Either one or both of these modifications can be performed on sialic acid to enhance the binding of DNA to mucin to form said mucin-DNA complex.

Furthermore, specific exoglycosidases can be used to expose specific carbohydrate groups on the mucin carbohydrate chains. This method can be used to tailor the properties of the mucin-DNA complex to the receptors present on specific target cells and to thus enhance endocytosis and transfection. For examples, lung cells recognize mannose in the terminal position whereas the liver's Kuffer cells recognize galactose in the terminal position. Still other cells may have sialic acid binding protein receptors (sialolectins).

The mucin used to form said mucin-DNA complex can consist of one or more different types of mucin molecules, each with the same or different types of modifications. The mucin-DNA complex, as described in the present invention thus offers a new tool for the transfection of cells and for the in vivo, or in vitro, delivery of DNA, RNA and other biomolecules into cells. The present invention can thus be used for gene therapy, for cell repair, cell modification or for the production of specific proteins or enzymes in specific cells. Said mucin-DNA complex is not limited by the size of DNA or other biomolecules used to form the complex with mucin.

The broader usefulness of the present invention may be illustrated by the following examples.

Example 1. Formation of a mucin-DNA complex.

Fluorescence tagged DNA was added to a mucin solution and the mixture was agitated by the use of a vortex for 1-2 minutes. The mucin was precipitated by the addition of isopropanol or other organic solvents. The resulting precipitate showed fluorescence whereas the remaining solution

Example 2. Stress induction on a newly formed mucin-DNA complex.

Fluorescence tagged DNA was added to a mucin solution and the mixture was agitated by the use of a vortex for 1-2 minutes. The mucin was precipitated by the addition of a gallnut extract, a natural product which has mucin precipitating properties. After precipitation the mucin-DNA complex showed fluorescence while the remaining solution showed no fluorescence, indicating that all of the DNA had combined with the mucin to form a mucin-DNA complex. The mucin-DNA complex was re-suspended in water and centrifuged for 1-2 minutes. Again, only the mucin-DNA complex showed fluorescence while the supernatant showed no fluorescence. Thus, the mucin-DNA complex formed, according to the present invention, is highly stable.

While a specific embodiment of the invention has been shown and described in detail to illustrate the application of the principles of the invention, it is understood that the invention may be embodied otherwise without departing from such principles and that various modifications, alternate constructions, and equivalents will occur to those skilled in the area given the benefit of this disclosure and the embodiment described herein, as defined by the appended claims.

WHAT IS CLAIMED IS

1. A mucin-DNA (deoxyribonucleic acid) complex formed by combining said mucin and said DNA in any configuration for the transport of said mucin-DNA complex into a cell using either *in vivo* or *in vitro* methods.

2. A mucin-biomolecules complex formed by combining said mucin and said biomolecules in any configuration for the transport of said mucin-biomolecules complex into a cell using either *in vivo* or *in vitro* methods.

3. Mucin as in claims 1 and 2, where said mucin can be a combination of one or more different types of mucin molecules obtained from any biological or non-biological source.

4. Mucin, as in claims 1 and 2, where said mucin can be in its native state or modified using any biological, chemical, enzymatic, heat-based or other means of modification.

5. Mucin, as in claims 1 and 2, where said mucin can contain sialic acid and its derivatives.

6. DNA, as in claims 1 and 2, where said DNA can be DNA or any other nucleic acid derived in a natural state, modified, or created synthetically and in any shape including linear, circular, single or double-stranded.

7. Biomolecules, as in claim 2, where said biomolecules may consist of one or more biomolecules from the group consisting of, but not limited to, DNA, RNA, nucleic acids, proteins, peptides, antibodies, glycolipids, glycoproteins, natural, synthetic and modified polymers, or any combination thereof.

8. Biomolecules, as in claim 2, where said biomolecules can be derived in a natural state, modified, or created synthetically.

9. A mucin-DNA complex as in claim 1 and mucin-biomolecules complex as in claim 2, where said complex can be purified by any chromatographic methods.

10. A mucin-DNA complex as in claim 1 and mucin-biomolecules complex as in claim 2, where said complex can be purified by any centrifugation methods.

11. A mucin-DNA complex as in claim 1 and mucin-biomolecules complex as in claim 2, where said mucin in said complex can undergo any modifications including, but not limited to, the addition, removal or alternation or carbohydrate or protein components or molecules of said mucin.

12. A mucin-DNA complex as in claim 1 and mucin-biomolecules complex as in claim 2, where said mucin in said complex can be modified to target specific cells as the targets of transfection.

13. A mucin-DNA complex as in claim 1 and mucin-biomolecules complex as in claim 2, where said complex can be used in applications including but not limited to gene therapy, cell repair, cell modification or the production of specific proteins or enzymes in specific cells.

SUMMARY OF THE INVENTION

In the present invention we describe a new method for the formation of a mucin-biomolecules complex, such as a mucin-DNA (deoxyribonucleic acid) complex and the application of such a complex for the transport of DNA, RNA (ribonucleic acid) and other biomolecules into cells. Transfection is the introduction of a DNA molecule into a eukaryotic cell, usually followed by the expression of one or more genes in the newly introduced DNA. The mucin-DNA complex described in the present invention can be used to perform transfection of DNA, as well as, the introduction of RNA and other larger biomolecules into cells. Since effective transfection, especially in *in vivo* systems is still limited by the methods currently available, the mucin-DNA complex, as described in the present invention, presents a novel and significantly improved method for performing transfection and ensuring the effective transmission of DNA into cells and the expression of genes in transfected DNA.

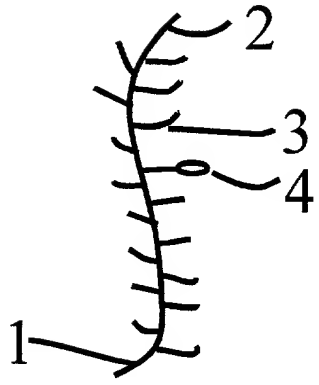

Fig. 1a

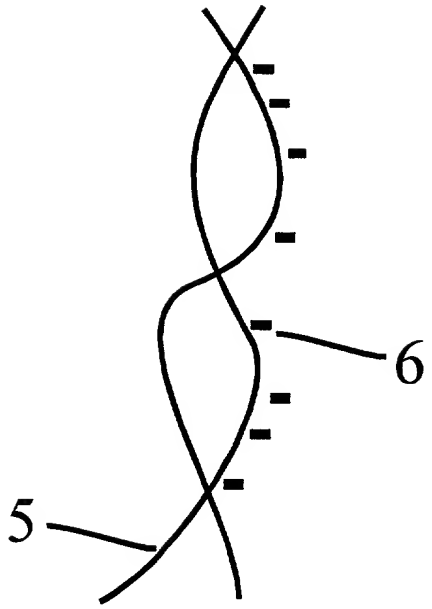

Fig. 1b

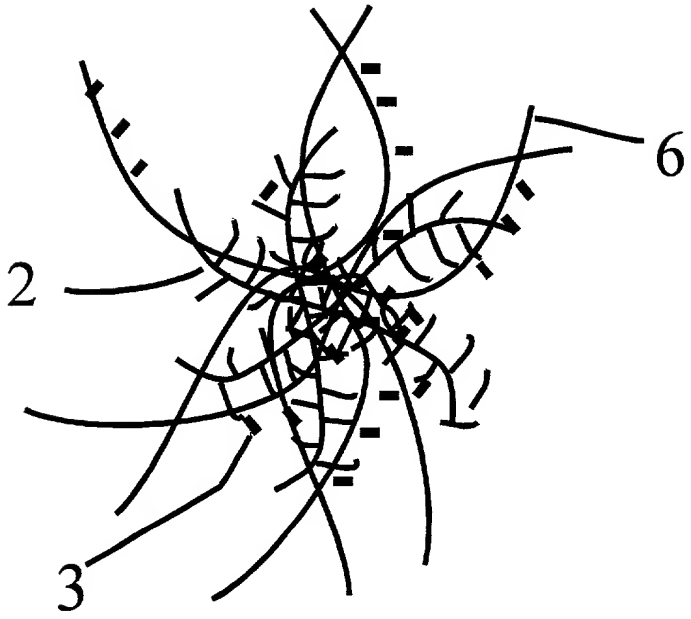

Fig. 2

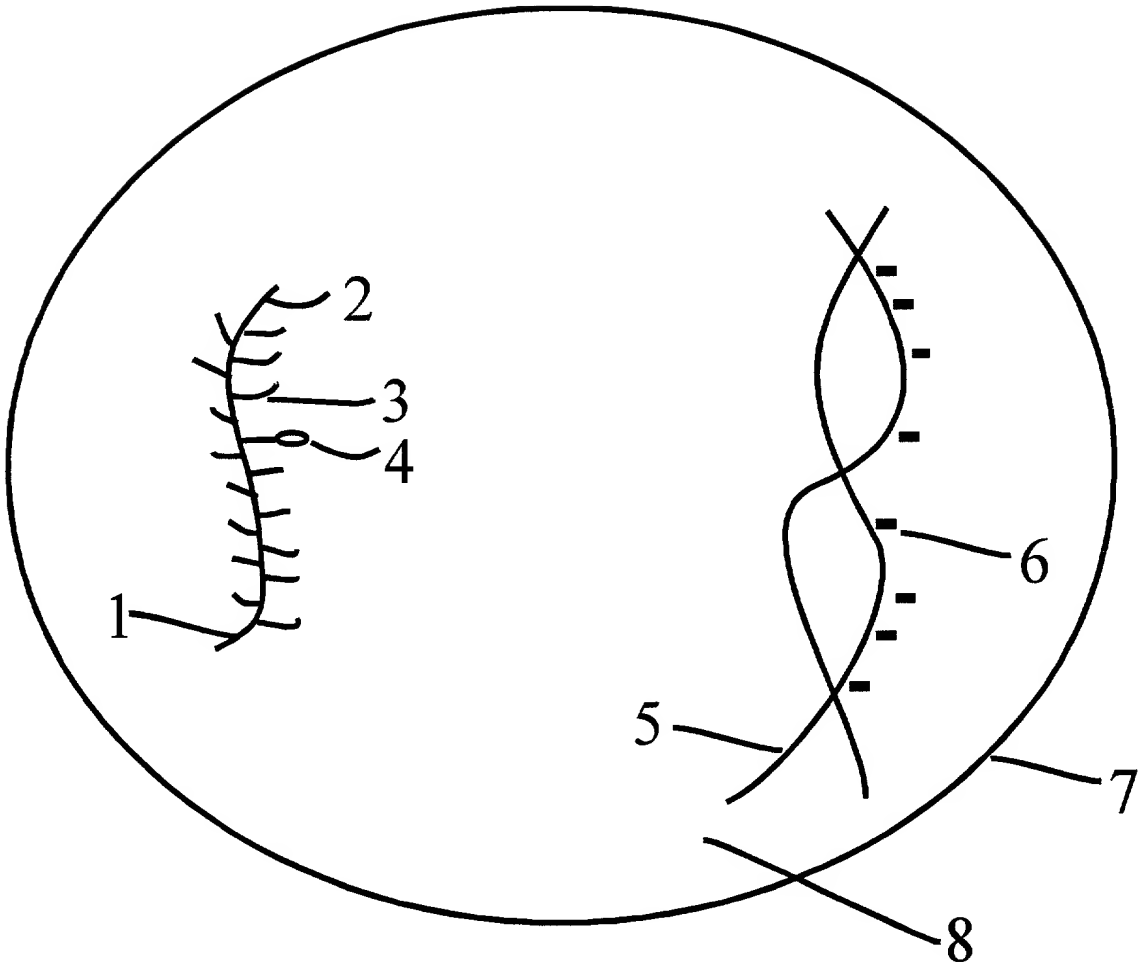

Fig. 3

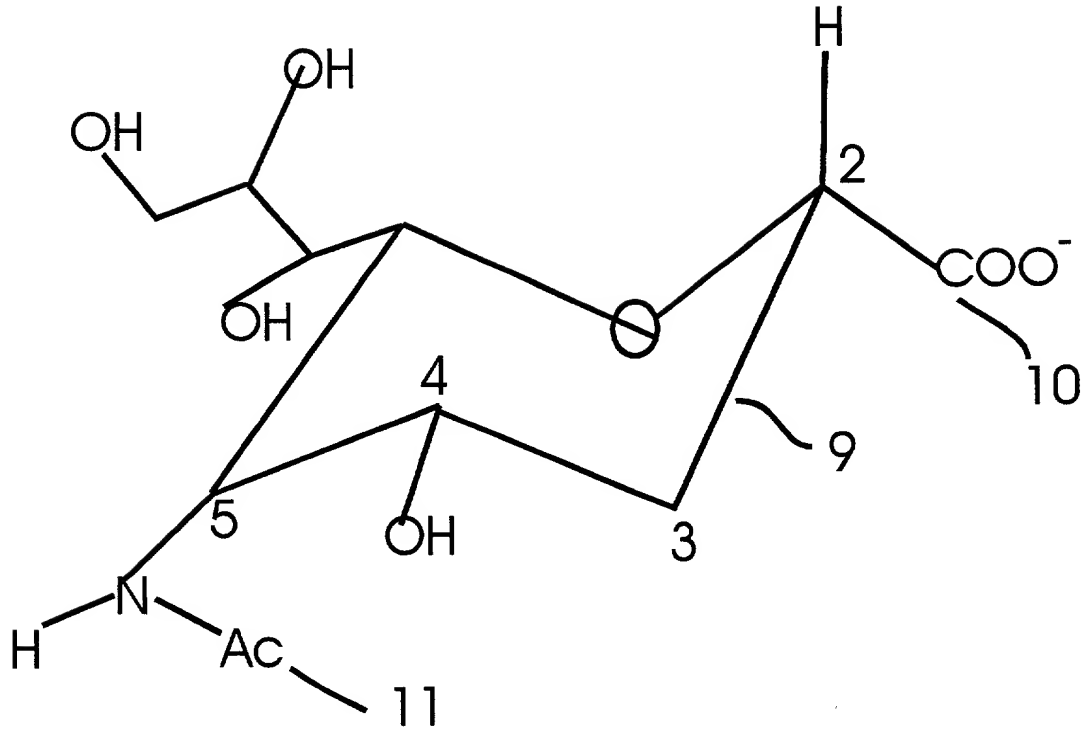

Fig. 4a

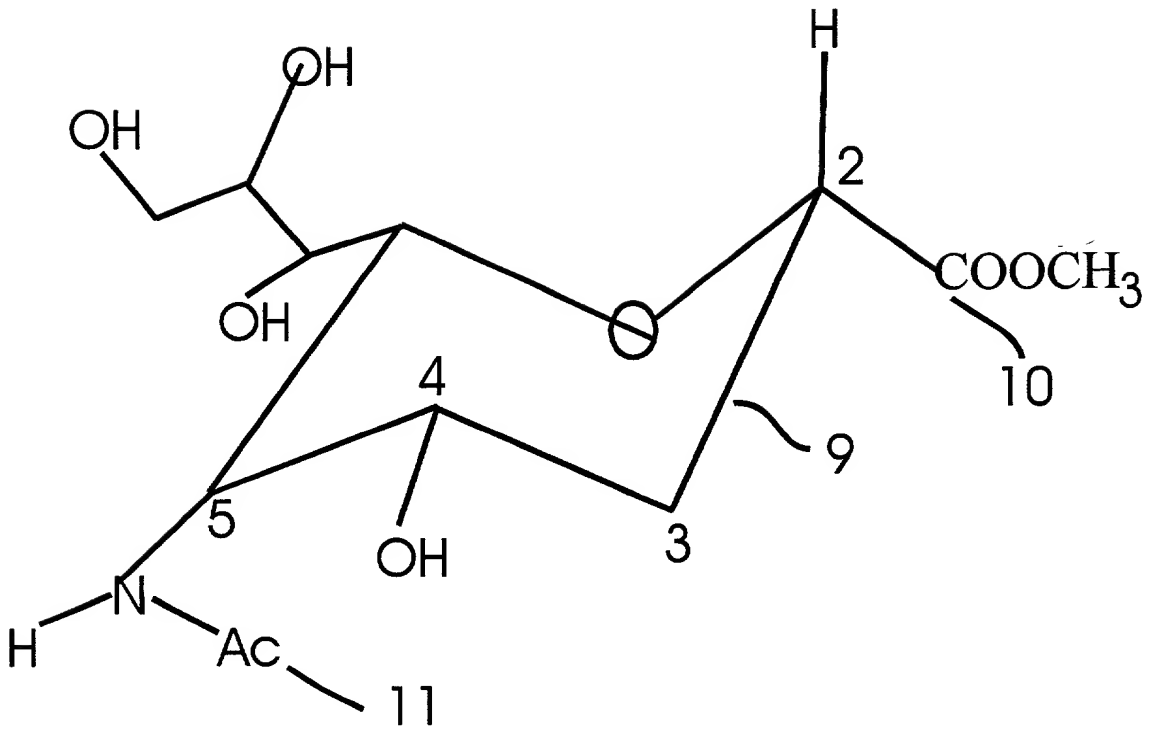

Fig. 4b

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

☒ Declaration Submitted with Initial Filing OR ☐ Declaration Submitted after Initial Filing

Attorney Docket Number	
First Named Inventor	ASHOK K. SHUKLA
COMPLETE IF KNOWN	
Application Number	
Filing Date	10/26/00
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled :

Mucin – Biomolecules Complex for Transfection

(Title of the invention)

the specification of which

☒ is attached hereto

OR

☐ was filed on (MM/DD/YYYY) ____ as United States Application Number or PCT International Application Number ____ and was amended on (MM/DD/YYYY) ____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code §119 (a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365 (a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto:

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

☐ Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.

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DECLARATION

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or §365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Name	Registration Number	Name	Registration Number

☐ Additional registered practitioner(s) named on a supplemental sheet attached hereto.

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Country	USA	Telephone	410 997 0301	Fax	410 997 0772

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

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Post Office Address							
City	WOODSTOCK	State	MD	Zip	21163	Country	USA

☒ Additional inventors are being named on supplemental sheet(s) attached hereto

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DECLARATION

ADDITIONAL INVENTOR(S) Supplemental Sheet

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

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Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name | Middle Initial | Family Name | Suffix e.g. Jr.

Inventor's Signature Date

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